3/4/06

Appln. No. 10/612,297 Filed: July 1, 2003 Page 2 of 16

## **Listing of Claims:**

1. (Currently Amended) A fluorescence energy transfer dye comprising having the Formula I:
--L1--D1--FETL--D2---L2-- (Formula I)

where:

- L1 is a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent;
- L2 is a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent;
- FETL is a fluorescence energy transfer linker comprising that is a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

D1 is a donor dye represented by the formula:

$$\mathbb{R}^4$$
 $\mathbb{R}^5$ 
 $\mathbb{R}^7$ 
 $\mathbb{R}^7$ 
 $\mathbb{R}^8$ 

DI

where:

X is O or C(R\*R\*\*), where R\* and R\*\* are independently lower alkyl or -CH2-Z;

R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

- R<sup>2</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>3</sup> to form an optionally substituted fused ring having 5 to 7 atoms;
- R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;
- R<sup>4</sup> is =O or OH, -N(R<sup>4</sup>'R<sup>4\*</sup>) or =N<sup>4</sup>(R<sup>4</sup>'R<sup>4\*</sup>), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused ring having 5 to 7 atoms,

where  $R^{4'}$  is H, lower alkyl or L1, and  $R^{4'}$  is H, lower alkyl or  $CH_{2'}Z$ ;

- R<sup>5</sup> is H, halo, Z, or is taken together with R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;
- R<sup>6</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

Appln. No. 10/612,297 Filed: July 1, 2003 Page 3 of 16

 $R^7$  is =0 or OH, -N( $R^7R^7$ ) or =N<sup>+</sup>( $R^7R^7$ ), or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused ring having 5 to 7 atoms,

where  $R^T$  is H, lower alkyl or L1, and  $R^T$  is H, lower alkyl or CH<sub>2</sub>-Z;

R<sup>8</sup> is halo, Z, or is taken together with R<sup>7</sup> and/or R<sup>9</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>8</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>8</sup> to form an optionally substituted fused ring having 5 to 7 atoms; and

Z is a group of the formula: -Z\*-Z¹- (linkage to L1, L2 or FETL), where:

Z\* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

 $Z^1$  is -C(O)-,  $-N(Z^2)$ -,  $-CH_2$ -O-,  $-CH_2$ -C(O)-,  $-CH_2$ -N( $Z^2$ )-,  $-CH_2$ -S-,  $-CH_2$ -S(O)-,  $-CH_2$ -S(O<sub>2</sub>)- or is absent; and

 $Z^2$  is H, C<sub>1</sub> to C<sub>8</sub> optionally substituted lower alkyl, or optionally substituted aryl; and D2 is an acceptor/reporter dye represented by formula D1 or by a formula of the group:

where:

at least one of R21 to R36 is joined to FETL,

n is zero, 1, 2 or 3;

R<sup>21</sup> and R<sup>30</sup> are independently –CH₂-Z, activated lower alkyl, or optionally substituted arlkylaralkyl;

R<sup>22</sup> to R<sup>29</sup> are independently H, SO<sub>3</sub>, or optionally substituted alkyl, or R<sup>22</sup> and R<sup>23</sup>, R<sup>23</sup> and R<sup>24</sup>, R<sup>24</sup> and R<sup>25</sup>, R<sup>26</sup> and R<sup>27</sup>, R<sup>27</sup> and R<sup>28</sup>, and/or R<sup>28</sup> and R<sup>29</sup> taken together form an optionally substituted fused ring having 6 atoms;

R<sup>31</sup> and R<sup>32</sup> are independently H, optionally substituted alkyl, aryl, or taken together form an optionally substituted fused ring having 6 atoms;

- R<sup>33</sup> to R<sup>36</sup> are independently H, SO<sub>3</sub>, optionally substituted alkyl, aryl, or R<sup>33</sup> and R<sup>34</sup>, R<sup>34</sup> and R<sup>35</sup>, and/or R<sup>35</sup> and R<sup>36</sup> taken together form an optionally substituted fused ring having 6 atoms; and
- Y is -O- or -N(Y¹)- where Y¹ is -CH₂-Z, activated lower alkyl, or optionally substituted arlkylaralkyl;

provided that at least one of R<sup>3</sup> to R<sup>8</sup> is Z where Z\* is optionally substituted aryl or optionally substituted heteroaryl, or a probe, target and/or support conjugate thereof.

- 2. (Currently Amended) The fluorescence energy transfer dye of Claim 1 having one or more of the following:
  - R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, 5-6 membered mono or 10-12 membered fused substituted aryl or hoteroafylheteroaryl, or Z;
  - R<sup>2</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>3</sup> to form an optionally substituted fused 6-membered aryl ring;
  - R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused 6-membered ring:
  - R<sup>4</sup> is =O or OH, -N(R<sup>4</sup>'R<sup>4</sup>') or =N<sup>+</sup>(R<sup>4</sup>'R<sup>4</sup>'), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused 6-membered ring;
  - R<sup>5</sup> is H, halo, Z, or is taken together with R<sup>4</sup> to form an optionally substituted fused 6membered ring:
  - R<sup>6</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused 6membered ring;
  - $R^7$  is =O or OH, -N( $R^7R^7$ ) or =N<sup>+</sup>( $R^7R^7$ ), or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused 6-membered ring;
  - R<sup>8</sup> is halo, Z, or is taken together with R<sup>7</sup> and/or R<sup>9</sup> to form an optionally substituted fused 6-membered ring;
  - R<sup>9</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>8</sup> to form an optionally substituted fused 6-membered aryl ring;
  - $R^4$  is  $-N(R^4R^4)$  or  $=N^*(R^4R^4)$  and  $R^7$  is  $-N(R^7R^7)$  or  $=N^*(R^7R^7)$  when X is  $C(R^*R^{**})$ ;
  - Z is a group of the formula: -Z\*-Z1- (linkage to L1, L2 or FETL), where:
    - Z\* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl,
    - $Z^1$  is -C(O)-,  $-N(Z^2)$ -,  $-CH_2$ -O-,  $-CH_2$ -C(O)-,  $-CH_2$ -N( $Z^2$ )-,  $-CH_2$ -S-,  $-CH_2$ -S(O)-,  $-CH_2$ -S(O<sub>2</sub>)- or is absent, and
    - Z<sup>2</sup> is H, or is C<sub>1</sub> to C<sub>8</sub> lower alkyl or aryl optionally substituted with SO<sub>3</sub>, COOH, NH<sub>2</sub>, CH<sub>2</sub>NH<sub>2</sub>, SH, or SCH<sub>3</sub>;

Appln. No. 10/612,297 Filed: July 1, 2003 Page 5 of 16

- FETL comprises a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;
- R<sup>21</sup> or R<sup>30</sup> is carboxy-naphthyl-methyl, mono- or di-*ortho*-substituted benzyl having an *ortho*-, *meta* or *para* carbonyl or activating group, or an activated C<sub>1</sub> to C<sub>6</sub> lower alkyl;
- R<sup>22</sup> to R<sup>29</sup> are independently H, SO<sub>3</sub> or optionally substituted alkyl, or R<sup>22</sup> and R<sup>23</sup>, R<sup>23</sup> and R<sup>24</sup>, R<sup>24</sup> and R<sup>25</sup>, R<sup>26</sup> and R<sup>27</sup>, R<sup>27</sup> and R<sup>28</sup>, and/or R<sup>28</sup> and R<sup>29</sup> taken together form a fused, sulfonated 6-membered aryl ring;
- R<sup>31</sup> and R<sup>32</sup> are independently H, optionally substituted alkyl, aryl, or taken together form a fused, sulfonated 6-membered carbocyclic or heterocyclic ring;
- R<sup>33</sup> to R<sup>36</sup> are independently H, SO<sub>3</sub>, optionally substituted alkyl, aryl, or R<sup>33</sup> and R<sup>34</sup>, R<sup>34</sup> and R<sup>35</sup>, and/or R<sup>35</sup> and R<sup>36</sup> taken together form a fused, sulfonated 6-membered carbocyclic or heterocyclic ring; and/or

at least one of R22 to R29 or R33 to R36 is SO3.

3. (Currently Amended) The fluorescence energy transfer dye of Claim 2 having one or more of the following:

R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, an optionally substituted *ortho*-benzoic acid, or Z;

R2 is H. halo or SO<sub>3</sub>:

R<sup>3</sup> is halo or Z;

 $R^4$  is =0 or OH, -N( $R^4$ ' $R^4$ ') or =N\*( $R^4$ ' $R^4$ ');

R<sup>5</sup> is H, halo or Z;

R<sup>6</sup> is H, halo or Z;

 $R^7$  is =0 or OH,  $-N(R^7R^7)$  or  $=N^+(R^7R^7)$ ;

.R<sup>8</sup> is Z;

R<sup>9</sup> is H, halo or SO<sub>3</sub>;

Z is a group of the formula: -Z\*-Z1- (linkage to L1, L2 or FETL), where:

Z\* is an optionally substituted aryl or heteroaryl of the group: phenyl, adamantlyadamantyl, norboranyl, biphenyl, naphthyl, furanyl, bifuranyl, thiophenyl, bithienyl, pyrrolyl, indolyl, isoindolyl, benzofuranyl, isobenzofuranyl, benzothiophenyl, benzothienyl, chromenyl and isochromenyl, and

 $Z^1$  is -C(O)-, -N(H)-,  $-CH_2$ -O-,  $-CH_2$ -C(O)-,  $-CH_2$ -N(H)-, or is absent;

R<sup>22</sup> to R<sup>29</sup> are independently H, SO<sub>3</sub> or optionally substituted alkyl, or R<sup>24</sup> and R<sup>25</sup> and/or R<sup>26</sup> and R<sup>27</sup> taken together form a fused, sulfonated 6-membered aryl ring;

 $\mbox{\ensuremath{R^{31}}}$  and  $\mbox{\ensuremath{R^{32}}}$  are independently H, optionally substituted alkyl or aryl; and/or

R<sup>33</sup> to R<sup>38</sup> are independently H, SO<sub>3</sub>, optionally substituted alkyl or aryl.

Appln. No. 10/612,297 Filed: July 1, 2003 Page 6 of 16

4. (Previously Amended) The fluorescence energy transfer dye of Claim 3 where R<sup>1</sup> is a group represented by Formula R1.1:

where:

R<sup>1a</sup> is H, halo or -C(O)O<sup>-</sup>; R<sup>1b</sup> is H, halo or -C(O)-(**linkage to** *L1*, *L2 or FETL*); R<sup>1c</sup> is halo; and r is 0, 1, 2 or 3.

- 5. (Currently Amended) The fluorescence energy transfer dye of Claim 1 where at least one of R<sup>3</sup> to R<sup>8</sup> is Z where Z\* is optionally substituted aryl selected from phenyl, adamantly adamantly, norboranyl, biphenyl and naphthyl.
- 6. (Original) The fluorescence energy transfer dye of Claim 5 where Z\* is phenyl and Z¹ is -C(O)-.
- (Currently Amended) The fluorescence energy transfer dye of Claim 1 where:
   R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, an optionally substituted *ortho*-benzoic acid, or
   Z;

R<sup>2</sup> is H, halo or SO<sub>3</sub>;

R<sup>3</sup> is halo or Z:

 $R^4$  is =0 or OH, -N( $R^4$ ' $R^4$ ') or = $N^*(R^4$ ' $R^4$ ');

R<sup>5</sup> is H, halo or Z;

R<sup>6</sup> is H, halo or Z;

 $R^{7}$  is =0 or OH,  $-N(R^{7}R^{7})$  or  $=N^{*}(R^{7}R^{7})$ ;

R<sup>8</sup> is Z;

R9 is H, halo or SO3;

Z is a group of the formula: -Z\*-Z¹- (linkage to L1, L2 or FETL), where:

Z\* is an optionally substituted aryl or heteroaryl of the group: phenyl, adamantlyadamantvl, norboranyl, biphenyl, naphthyl, furanyl, bifuranyl, thiophenyl, bithienyl, pyrrolyl, indolyl, isoindolyl, benzofuranyl, Appln. No. 10/612,297 Filed: July 1, 2003 Page 7 of 16

isobenzofuranyl, benzothiophenyl, benzothienyl, chromenyl and isochromenyl, and

 $Z^1$  is -C(O)-, -N(H)-,  $-CH_2$ -O-,  $-CH_2$ -C(O)-,  $-CH_2$ -N(H)-, or is absent;

R<sup>21</sup> or R<sup>30</sup> is carboxy-naphthyl-methyl, mono- or di-*ortho*-substituted benzyl having an *ortho*-, *meta*- or *para*- carbonyl or activating group, or an activated C<sub>1</sub> to C<sub>6</sub> lower alkyl;

R<sup>22</sup> to R<sup>29</sup> are independently H, SO<sub>3</sub> or optionally substituted alkyl, or R<sup>24</sup> and R<sup>25</sup> and/or R<sup>26</sup> and R<sup>27</sup> taken together form a fused, sulfonated 6-membered aryl ring;

R<sup>31</sup> and R<sup>32</sup> are independently H, optionally substituted alkyl or aryl; and

R<sup>33</sup> to R<sup>36</sup> are independently H, SO<sub>3</sub>, optionally substituted alkyl or aryl.

8. (Previously Amended) The fluorescence energy transfer dye of Claim 1 where FETL is represented by a formula of the group:

where:

 $R^{10}$  is -C(O)-, -N(H)- $CH_{2}$ -, -S-C(O)-, -O-C(S)- or -S- $CH_{2}$ -; and  $R^{11}$  is -C(O)-,  $-CH_{2}$ -N(H)-; -C(O)-S-, -C(S)-O- or  $CH_{2}$ -S-;

where:

p is independently 0, 1, 2 or 3; and

Appln. No. 10/612,297 Filed: July 1, 2003 Page 8 of 16

where:

q is zero or 1;

R<sup>12</sup> is H, halo, or optionally substituted-alkyl, -alkenyl, -alkynyl or -aryl;

R<sup>13</sup> is H, SO<sub>3</sub> or optionally substituted-alkyl, -alkenyl, -alkynyl or -aryl; and

R<sup>14</sup> is a secondary or tertiary amine or heterocyclyl, particularly N(H) or piperazine.

9. (Currently Amended) The fluorescence energy transfer dye of Claim 8 where FETL is FETL1 and:

$$R^{10}$$
 is  $-C(O)$ - or  $-N(H)$ - $CH_2$ -; and  $R^{11}$  is  $-C(O)$ - or  $-CH_2$ - $N(H)$ -.

10. (Currently Amended) A compound represented by the formula:

$$R^4$$
 $R^5$ 
 $R^6$ 
 $R^7$ 
 $R^8$ 
 $R^8$ 

D1

where:

X is O or C(R\*R\*\*), where R\* and R\*\* are independently lower alkyl or -CH2-Z;

R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

R<sup>2</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>3</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>4</sup> is =O or OH, -N(R<sup>4</sup>'R<sup>4</sup>') or =N<sup>+</sup>(R<sup>4</sup>'R<sup>4</sup>'), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused ring having 5 to 7 atoms,

where R<sup>4</sup> is H, lower alkyl or L1, and R<sup>4</sup> is H, lower alkyl or CH<sub>2</sub>-Z;

R<sup>5</sup> is H, halo, Z, or is taken together with R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>6</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

 $R^7$  is =0 or OH, -N( $R^7R^7$ ) or =N<sup>+</sup>( $R^7R^7$ ), or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused ring having 5 to 7 atoms,

Appln. No. 10/612,297 Filed: July 1, 2003 Page 9 of 16

where  $R^{T}$  is H, lower alkyl or L1, and  $R^{T}$  is H, lower alkyl or  $CH_{2}$ -Z;

- R<sup>8</sup> is halo, Z, or is taken together with R<sup>7</sup> and/or R<sup>9</sup> to form an optionally substituted fused ring having 5 to 7 atoms;
- R<sup>9</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>8</sup> to form an optionally substituted fused ring having 5 to 7 atoms; and
- Z is a group of the formula: -Z\*-Z1- (linkage to L1, L2 or FETL), where:
  - Z\* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;
  - $Z^1$  is -C(O)-,  $-N(Z^2)$ -,  $-CH_2$ -O-,  $-CH_2$ -C(O)-,  $-CH_2$ -N( $Z^2$ )-,  $-CH_2$ -S-,  $-CH_2$ -S(O)-,  $-CH_2$ -S(O<sub>2</sub>)- or is absent; and
  - Z<sup>2</sup> is H, C<sub>1</sub> to C<sub>8</sub> optionally substituted lower alkyl, or optionally substituted aryl;
  - L1 being a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent;
  - L2 being a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent; and
  - FETL being a fluorescence energy transfer linker comprising that is a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

provided that at least one of R<sup>3</sup> to R<sup>8</sup> is Z, where Z\* is optionally substituted aryl or optionally substituted heteroaryl, or a FETL, probe, target and/or support conjugate thereof.

- 11. (Currently Amended) The compound of Claim 10 having one or more of the following: R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, 5-6 membered mono or 10-12 membered fused substituted aryl or heteroafylheteroaryl, or Z;
  - R<sup>2</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>3</sup> to form an optionally substituted fused 6-membered aryl ring;
  - R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused 6-membered ring;
  - R<sup>4</sup> is =O or OH, -N(R<sup>4</sup>'R<sup>4</sup>') or =N<sup>+</sup>(R<sup>4</sup>'R<sup>4</sup>'), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused 6-membered ring;
  - R<sup>5</sup> is H, halo, Z, or is taken together with R⁴ to form an optionally substituted fused 6-membered ring;
  - R<sup>8</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused 6membered ring;

Appln. No. 10/612,297 Filed: July 1, 2003 Page 10 of 16

 $R^7$  is =0 or OH, -N( $R^TR^T$ ) or =N<sup>+</sup>( $R^TR^T$ ), or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused 6-membered ring;

R<sup>8</sup> is halo, Z, or is taken together with R<sup>7</sup> and/or R<sup>9</sup> to form an optionally substituted fused 6-membered ring;

R<sup>9</sup> is H, halo, SO<sub>3</sub><sup>-</sup>, or is taken together with R<sup>8</sup> to form an optionally substituted fused 6-membered aryl ring;

 $R^4$  is  $-N(R^4R^4)$  or  $=N^*(R^4R^4)$  and  $R^7$  is  $-N(R^7R^7)$  or  $=N^*(R^7R^7)$  when X is  $C(R^*R^{**})$ ; and/or Z is a group of the formula:  $-Z^*-Z^{1-}$  (linkage to L1, L2 or FETL), where:

Z\* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl,

 $Z^1$  is -C(O)-,  $-N(Z^2)$ -,  $-CH_2$ -O-,  $-CH_2$ -C(O)-,  $-CH_2$ -N( $Z^2$ )-,  $-CH_2$ -S-,  $-CH_2$ -S(O)-,  $-CH_2$ -S(O<sub>2</sub>)- or is absent, and

 $Z^2$  is H, or is C<sub>1</sub> to C<sub>8</sub> lower alkyl or aryl optionally substituted with SO<sub>3</sub>, COOH, NH<sub>2</sub>, CH<sub>2</sub>NH<sub>2</sub>, SH, or SCH<sub>3</sub>.

12. (Currently Amended) A fluorescence energy transfer dye <u>conjugate</u> represented by Formula II:

where:

Probe is a polynucleotide, antibody, triglyceride, low density lipoprotein or lectin;

L1 is a link that is a chemical bond for attachment to the Probe;

FETL is a fluorescence energy transfer linker comprising that is a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

low affinity false target is an analyte for the Probe disposed on FETL to deactivate, block or otherwise prevent coupling of FETL to a corresponding D2 or D1 until said low affinity false target is displaced by a higher affinity true target;

D1 is a donor dye represented by the formula:

$$\mathbb{R}^4$$
 $\mathbb{R}^5$ 
 $\mathbb{R}^6$ 
 $\mathbb{R}^7$ 
 $\mathbb{R}^7$ 

Appln. No. 10/612,297 Filed: July 1, 2003 Page 11 of 16

#### where:

X is O or C(R\*R\*\*), where R\* and R\*\* are independently lower alkyl or -CH2-Z;

·R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

R<sup>2</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>3</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>4</sup> is =O or OH, -N(R<sup>4</sup>'R<sup>4</sup>') or =N<sup>+</sup>(R<sup>4</sup>'R<sup>4</sup>'), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused ring having 5 to 7 atoms,

where  $R^{4'}$  is H, lower alkyl or L1, and  $R^{4'}$  is H, lower alkyl or  $CH_{2'}Z$ ;

R<sup>5</sup> is H, halo, Z, or is taken together with R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>6</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

 $R^7$  is =0 or OH, -N( $R^7R^7$ ) or = $N^+(R^7R^7)$ , or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused ring having 5 to 7 atoms,

where  $R^{T}$  is H, lower alkyl or L1, and  $R^{T}$  is H, lower alkyl or  $CH_{2}$ -Z;

R<sup>8</sup> is halo, Z, or is taken together with R<sup>7</sup> and/or R<sup>9</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>8</sup> is H, halo, SO<sub>3</sub>, or is taken together with R<sup>8</sup> to form an optionally substituted fused ring having 5 to 7 atoms; and

Z is a group of the formula: -Z\*-Z1- (linkage to L1, L2 or FETL), where:

Z\* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

 $Z^1$  is -C(O)- , -N( $Z^2$ )- , -CH<sub>2</sub>-O- , -CH<sub>2</sub>-C(O)- , -CH<sub>2</sub>-N( $Z^2$ )- , -CH<sub>2</sub>-S- , -CH<sub>2</sub>-S(O)- , -CH<sub>2</sub>-S(O<sub>2</sub>)- or is absent; and

 $Z^2$  is H, C<sub>1</sub> to C<sub>8</sub> optionally substituted lower alkyl, or optionally substituted aryl; and D2 is an acceptor/reporter dye represented by formula D1 or by a formula of the group:

Appln. No. 10/612,297 Filed: July 1, 2003 Page 12 of 16

where:

at least one of R21 to R36 is joined to FETL,

n is zero, 1, 2 or 3;

R<sup>21</sup> and R<sup>30</sup> are independently –CH₂-Z, activated lower alkyl, or optionally substituted arlkylaralkyl;

R<sup>22</sup> to R<sup>29</sup> are independently H, SO<sub>3</sub>, or optionally substituted alkyl, or R<sup>22</sup> and R<sup>23</sup>, R<sup>23</sup> and R<sup>24</sup>, R<sup>24</sup> and R<sup>25</sup>, R<sup>26</sup> and R<sup>27</sup>, R<sup>27</sup> and R<sup>28</sup>, and/or R<sup>28</sup> and R<sup>29</sup> taken together form an optionally substituted fused ring having 6 atoms; and

R<sup>31</sup> and R<sup>32</sup> are independently H, optionally substituted alkyl, aryl, or taken together form an optionally substituted fused ring having 6 atoms;

R<sup>33</sup> to R<sup>36</sup> are independently H, SO<sub>3</sub>, optionally substituted alkyl, aryl, or R<sup>33</sup> and R<sup>34</sup>, R<sup>34</sup> and R<sup>35</sup>, and/or R<sup>35</sup> and R<sup>36</sup> taken together form an optionally substituted fused ring having 6 atoms; and

Y is -O- or -N(Y¹)- where Y¹ is -CH<sub>2</sub>-Z, activated lower alkyl, or optionally substituted aralkyl.

- 13. (Currently Amended) A fluorescence energy transfer assay for determining the presence of a target site in a substance requiring no pre-analysis purification for removal of unbound dye, comprising the steps of:
  - (a) contacting a substance to be tested and a target site specific Probe-L1-(D1 or D2)-FETL-(low affinity false target) conjugate of Claim 12 in a suitable assay vessel under conditions suitable for preferential binding of the Probe to the target site, as opposed to the low affinity false target;
  - (b) introducing into the vessel a second D2 or D1 fluorescence energy transfer dye having an activated site for coupling to the FETL-into-the vessel, under conditions suitable for

Appln. No. 10/612,297 Filed: July 1, 2003 Page 13 of 16

coupling to FETL where the low affinity false target is not bound to the Probe, wherein the second dye is the other of the D1 or D2 found in the conjugate;

- (c) causing D1 to absorb energy; and
- (d) measuring the level of D2 emission, wherein emission from D2 upon absorption of energy by D1 indicates the presence of the target site in the substance.

14-16. (Cancelled)



- 17. (Currently Amended) A proximity assay comprising the steps of :
  - (a) contacting a substance to be tested and a target-site-specific donor dye in a suitable assay vessel;
  - (b) introducing a target-site-specific fluorescence energy transfer reporter dye conjugate of Claim 1-19 into the vessel, where said reporter dye's target is either spatially proximate to said donor dye target or specific for a given target to be tested for spatial proximity to said donor dye target, and said reporter dye's energy absorption spectra overlaps the emission spectra of said donor dye;
  - (c) causing the donor dye to absorb energy; and
  - (d) measuring the level of reporter dye emission, wherein emission from the reporter dye upon absorption of energy by the donor dye indicates the proximity of the donor dye target and the reporter dye target in the substance.



(Currently Amended) The proximity assay of Claim 17 wherein:

- step (b) further comprises introducing two or more target-site-specific fluorescence energy transfer reporter dye conjugates of Claim 4-19 into the vessel, said reporter dyes having energy absorption spectra overlapping the emission spectra of said donor dye, distinct emission spectra, and having different targets to be tested for spatial proximity to said donor dye target; and
- step (d) further comprises measuring the level of reporter dye emission at the wavelengths characteristic of said reporter dyes, wherein emission characteristic of each reporter dye upon absorption of energy by the donor dye indicates the respective proximity of the donor dye target to each corresponding reporter dye target in the substance.



19. (New) A conjugate comprising the dye of claim 1 conjugated to a probe, a target, and/or a support.



20. (New) A conjugate comprising the compound of claim 10 conjugated to a probe, an FETL, a target, and/or a support.

Claims 1-13, 17-18 are pending in this application.

Claims 14-16 are cancelled.

#### Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-13, 17-18 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The structures represented by L1, L2 and "FETL" must be shown in every occurrence in the claims. Applicant cannot claim all or any known L1, L2 and FETL compounds that may be applicable in the instant invention because the specification fails to state that all or any known L1, L2 and FETL compounds is/are applicable in the invention. Therefore, the specification fails to provide adequate support for the claims. Additionally, FETL is a critical element of the invention and therefore must be disclosed in the claims. See the MPEP. This is not possible to ascertain the structures and/or identities of L1 and L2. If L1 and L2 are chemical bonds it must be so stated in the specification and the claims. By adding claim 8 to claim 1 define L1 and L2 as bonds the rejection would be overcome.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 1-13, 17-18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

For reasons set forth above under 35 USC 112, first paragraph, claims 1-13, 17-18 are indefinite.

The term "comprising" in claim 1, line 6 and claim 10, line 37 renders claims 1-7 10-13, 17-18 indefinite. The term is an open-ended term, which cannot be used in reference to a compound. By replacing the term with "is" the rejection would be overcome.

The term "into" in claim 13, line 9, is idiomatic and therefore, the claim is indefinite. By replacing the term with "in" the rejection would be overcome.

Applicant's arguments filed 8/16/05 have been fully considered but they are not persuasive. Applicant contends that FETL is not a critical element of the broadest claim, applicant is not limited to the preferred embodiments and that FETL1-4 are examples. This is not persuasive because a critical element of an invention is an element without which the invention would not work as designed. In the instant invention without FETL the invention would not work as claimed. While applicant is not limited to the preferred examples, the metes and bounds of each claim is a statutory requirement. Applicant should also note that "Exemplification is not an explicit definition." The specification must set forth the definition explicitly and clearly, with reasonable clarity, deliberateness and precision, *Teleflex Inc. v. Ficosa North Am Corp.*, 63 USPQ2d 1374, (Fed. Cir. 2002), *Rexnord Corp. v. Laitram Corp.*, 60 USPQ2d 1854 (Fed. Cir. 2001).

Application/Control Number: 10/612,297

Art Unit: 1626

# Figure Drawing

The Datasheet in this application indicates four drawing sheets were filed. However, none is in the case as at this time. Applicant must file new copies.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

### Telephone Inquiry

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Taofiq A. Solola, PhD., JD., whose telephone number is (571) 272-0709.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mr. Joseph McKane, can be reached on (571) 272-0699. The fax phone number for this Group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (571) 272-1600.

Page 5

TAOFIQ SOLOLA PRIMARY EXAMINER

**Group 1626** 

September 29, 2005